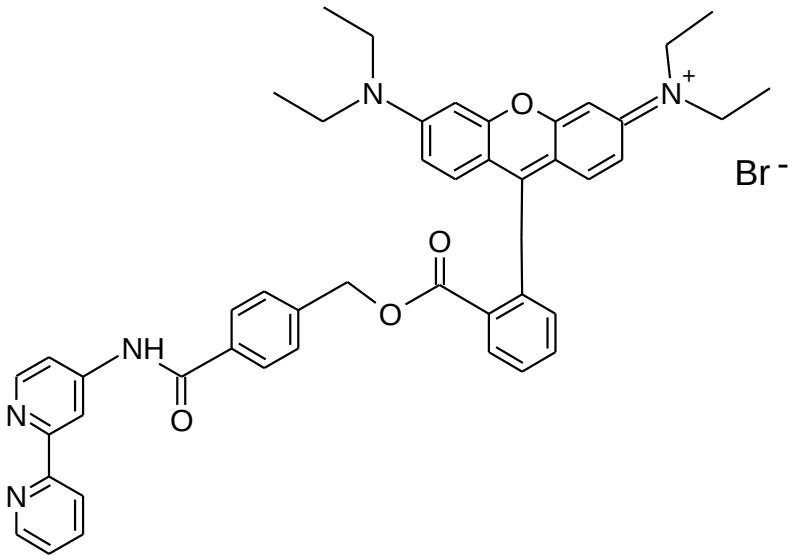
# PRODUCT INFORMATION

## RDA

**Cat. No. ME042.1 (1 mg)**
**Cat. No. ME042.2 (5 mg)**

**Rhodamine B-[(2,2´-bipyridine-4-yl)-aminocarbonyl]benzyl ester**




- $Fe^{2+}$ specific fluorescent “sensor”
- Mitochondria specific
- Determination of the mitochondrial chelatable iron pool
- Assessment of mitochondrial iron uptake
- Assessment of alterations of the mitochondrial chelatable iron pool under pathological conditions
- Assessment of the contribution of mitochondrial chelatable iron to physiological and pathological cellular processes
- Assessment of iron reduction

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## Selective Determination of Mitochondrial Chelatable Iron in Viable Cells with Fluorescent “Iron Sensors” RPA and RDA

Iron is essential for many biological processes, but is also detrimental as it fosters the generation of highly destructive oxygen species. Mitochondrial chelatable iron is considered to contribute to several human diseases. In the past, attempts to determine the intra-mitochondrial pool of chelatable iron were problematic, due to insufficient mitochondrial accumulation or uniform cellular distribution of iron-selective indicators [3]. The fluorescent non-toxic “iron sensors” RPA and RDA are the first sensors, which allow to determine this iron pool specifically in single intact mitochondria. They are new tools to assess the function of labile (“redox-active”) iron e.g. in processes of cell and tissue injury where free radicals are considered to be involved.

## Product Information

RDA is used as a selective, high quantum yield fluorescence marker for $Fe^{2+}$ in biological samples. It has a slightly lower affinity for iron than RPA. The cationic fluorophore of the membrane-permeable compound allows the observation by e.g. fluorescence microscopy and, based on the negative membrane potential of mitochondria, the targeting especially into mitochondria of viable cells. In a cell-free system, RDA fluorescence ($\lambda_{max}$ 598 nm) is strongly and stoichiometrically (3:1) quenched by $Fe^{2+}$ ions.

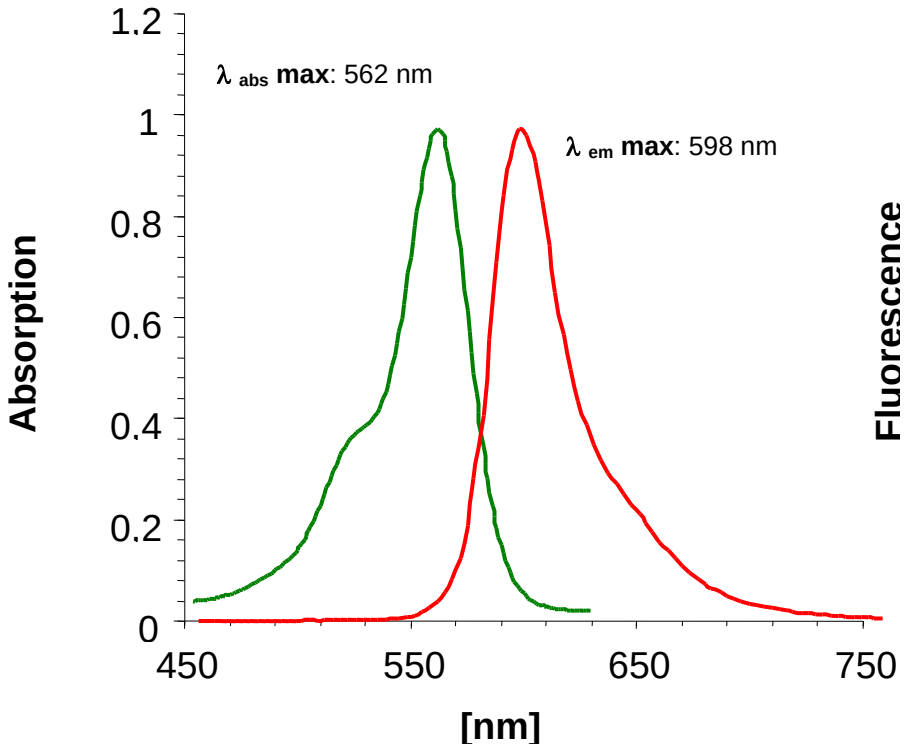


**Fig. A:** Absorbance and emission spectra for RDA (10 µM RDA in a “simple buffered solution”: 2 mM ascorbate, 0.18% SDS and 10 mM Tris/HCl at pH 8.2) in a cell-free system.

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RDA selectively accumulates in the mitochondria of cells, e.g. of cultured hepatocytes [(2)]. Intra-mitochondrial RDA fluorescence is quenched when iron is added to cells in a membrane-permeant form. It increases when the mitochondrial chelatable iron available is experimentally decreased after addition of membrane-permeant transition metal chelators pyridoxal isonicotinoyl hydrazone and 1,10-phenanthroline. This increase of RDA fluorescence allows to specifically quantify mitochondrial chelatable iron in viable cells using *ex situ* calibration as described for RPA [(1)].

## Application in a cellular system

RDA can be used in an identically manner as RPA [(1)]. Viable cells, cultivated on coverslips for example are incubated with RDA (0.1-0.5 µM, prepared from stock solutions of 1-5 mM in DMSO) for 10-20 min at 37°C in HBSS ("Hanks balanced salt solution"). Cells are washed subsequently three times with dye-free HBSS. To foster mitochondrial selectivity, cells should be transferred after RDA loading to a second (indicator-free) Pentz chamber and incubated another 15 min. at 37°C. Cells should now be covered with an appropriate medium (e.g. HBSS or L-15 medium for hepatocytes) at 37°C. Intra-mitochondrial RDA fluorescence is determined using e.g. quantitative laser scanning microscopy (semiquantitative measurements are also possible using conventional fluorescence microscopy or a microtiter plate reader). The red fluorescence of RDA is excited at $\lambda_{exc.}$ = 562 nm and collected through a long-pass filter near $\lambda_{exc.}$ = 598 nm. Scanning parameters for quantitative and qualitative measurements depend on the microscope system used for the experiments. The intra-mitochondrial level of chelatable iron can be manipulated after establishing the baseline (5-10 min) by addition of $FeCl_3$/8-hydroxyquinoline complex (5-15 µM) or membrane permeant iron chelators, e.g. PIH (pyridoxal isonicotinoyl hydrazone, 2mM) or 1,10-phenanthroline (2 mM). For control measurements, parallel cultures are loaded with the iron-insensitive control RPAC (Rhodamine B-[(phenanthren-9-yl)aminocarbonyl]benzyl ester) containing the same fluorophore. The same loading conditions should be used as described above.

## *Ex situ* calibration: a) mitochondrial RDA concentrations

The *ex situ* calibration of intra-mitochondrial RDA concentrations is determined by comparing the mitochondrial fluorescence (arbitrary units) after "dequenching" with PIH (2 mM) with the fluorescence of RDA standards (5-80 µM) dissolved in a "mitochondrial medium" (see ref. 1) supplemented with 0.18% SDS. In order to obtain a calibration curve, 100 µl portions of medium with known RDA concentrations are placed on the same coverslips as used in cellular experiments. The same focal plane and laser scanning parameters used for the quantitative cellular fluorescence measurements are used in the cell-free *ex situ* calibrations.

## *Ex situ* calibration: b) $Fe^{2+}$ induced quenching of RDA fluorescence

1 ml of "mitochondrial medium" (see ref. 1) supplemented with 0.18% SDS is transferred into 1.5 ml tubes, incubated at 37°C. RDA (20-80 µM) is added. Known concentrations of FAS (ferrous ammonium sulphate/citric acid trisodium salt dihydrate) from a freshly prepared stock solution (1 mM) with 20 mM ascorbate are added. The RDA/$Fe^{2+}$ complex (3:1) is formed during approximately 2 min incubation time. The calibration curve is obtained by measuring the RDA fluorescence with the same laser scanning parameters used for the quantitative cellular fluorescence measurements.

## Product Data

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|---|---|
| product name: | RDA |
| chemical name: | Rhodamine B-[(2,2´-bipyridine-4yl)aminocarbonyl]-benzyl ester |
| molecular formula: | $C_{46}H_{44}N_5O_4Br$ |
| molecular weight: | 810,80 g/mol |
| absorption maximum: | $\lambda_{max}$ 562 nm |
| emission maximum: | $\lambda_{max}$ 598 nm |
| stability: | 4°C, stored dry and protected from light |
| appearance: | purple solid |
| purity: | > 97% ($^1$H NMR, 500 MHz) |
| quenching stoichiometry: | RDA/$Fe^{2+}$: 3:1 [mol/mol] |
| in vitro toxicity: | non toxic |

## Considerations for Use

The product is used as a selective, high quantum yield fluorescence marker for $Fe^{2+}$ in biological samples, especially in mitochondria of viable cells. RDA has a slightly lower affinity to iron compared to RPA. Measurements can be performed by fluorescence spectroscopy, fluorescence plate readers, FACS, video microscopy and laser scanning microscopy. 1-5 mM stock solutions of RDA in DMSO can be prepared and aliquots should be kept at –20°C. When stored properly at –20°C, the solutions can be used for at least 2 – 3 months.

## Literature


1. Selective determination of mitochondrial chelatable iron in viable cells with a new fluorescent sensor. F. Petrat et al. *Biochem. J.* (2002) 362, 137-147
2. Cold-induced apoptosis of hepatocytes: mitochondrial permeability transition triggered by nonmitochondrial chelatable iron. U. Rauen et al. Free Radical Biology & Medicine, Vol. 35, No. 12, pp. 1664-1678, 2003
3. The chelatable iron pool in living cells: A methodically defined quantity. F. Petrat et al. Biol. Chem., Vol. 383, pp. 489-502, 2002
4. Assessment of chelatable mitochondrial iron by using mitochondrion-selective fluorescent iron indicators with different iron-binding affinities. U. Rauen et al. ChemBioChem 2007, 8, 341-352
5. Oxidative inactivation of mitochondrial Aconitase results in iron and $H_2O_2$-mediated neurotoxicity in rat primary mesencephalic cultures. David Cantu *et al*. PlosOne, September 2009, Vol 4, Issue 9, p 1-9


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